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BIODETERIORATION OF CEMENTEOUS MATRIX BY FUNGI**BIODETERIORATION D'UNE MATRICE CIMENTAIRE PAR LES CHAMPIGNONS**Virginie WIKTOR¹, Philippe GROSSEAU¹, René GUYONNET¹, Eric GARCIA-DIAZ²¹ Ecole Nationale Supérieure des Mines de Saint-Etienne, Saint-Etienne, France² Ecole des Mines de Douai, Douai, France

ABSTRACT – Biodeterioration mechanisms are complex and not yet absolutely understood. To control and to act efficiently against deterioration by fungi, it is necessary to have a better understanding of those mechanisms. The aim of this paper is to present different stages taken into account for the setting up of a laboratory accelerated test of biodeterioration of cementeous matrix by fungi. Indeed in natural environment, biodeterioration takes place after several years. Incubation tests performed on non weathered and weathered specimens underline the importance of pH surface on fungi development. Best solution for the moment appears to be carbonation and leaching of specimens for matrix weathering and inoculation with spores suspension of specimen covered by a thin agar-agar film.

RÉSUMÉ – Les mécanismes de la biodeterioration sont complexes et pas encore totalement compris. Afin de contrôler et d'agir efficacement contre la biodétérioration par les champignons, il est nécessaire d'avoir une meilleure compréhension de ces mécanismes. Le but de cet article est de présenter les différents étapes intervenant dans la mise au point d'un test accéléré de laboratoire sur la biodétérioration d'une matrice cimentaire par les champignons. En effet, en environnement naturel, la biodétérioration s'établit après plusieurs années. Les tests d'incubations réalisés sur des éprouvettes non vieilles et vieilles soulignent l'importance du pH de surface sur le développement fongique. Actuellement la meilleure solution semble être pour le vieillissement de la matrice : la carbonatation et lixiviation des éprouvettes, et l'inoculation par une suspension de spore d'éprouvettes recouvertes d'un fin film de gélose.

1. Introduction

Micro-organisms – bacteria and cyanobacteria, fungi, algae, and lichens – are liable to grow on building materials: concrete, wall plastering, stone.... They are liable to degrade material properties: from an alteration of aesthetic aspect to a reduction of its service life. Biodeterioration mechanisms are complex and not yet absolutely understood. The biodeterioration is enhanced by environmental factors such as freeze/thaw cycles or pollution (Warscheid, 2000).

Fungi are among the most harmful organisms associated to biodeterioration of organic and inorganic materials. Their occurrence on the stones is reported to be combined not only with aesthetical spoiling of the monuments, due to color changes and black spots, but also with a strong evidence that these organisms are causing crater shaped lesions, chipping and exfoliation of the rock surface combined with the loss of materials (Urzi et al. 1995, Urzi et al. 2000b). The black fungi such as *Alternaria* have the capacity to settle on the surface of

rocks, to attach firmly and to penetrate deeper into the rock. The major way of attack and penetration of this group of rock infecting fungi is physical (Diakumaku et al. 1995). Acidogenic fungi such as *Aspergillus*, *Penicillium* produce different organic acids, such as oxalic, gluconic, malic, citric, etc., in low and normal glucose concentrations (de la Torre et al. 1991). These acids form calcium salts or act as chelating agents of mineral cations favouring the biodeterioration process (Berthelin 1983).

To control and to act efficiently against deterioration by fungi, it is necessary to have a better understanding of those mechanisms.

The aim of this paper is to present different stages taken into account for the setting up of a laboratory accelerated test of biodeterioration by fungi. Indeed in natural environment, biodeterioration takes place after several years. The most complex stage is to accelerate fungi development on specimens in order to reduce this time to observe biodeterioration. Two fungal strains were chosen for the study: (i) *Alternaria alternata*, responsible of physical deterioration, and (ii) *Aspergillus niger*, an acidogenic fungi responsible of chemical deterioration. Several parameters are studied: firstly physico-chemical parameters which accelerate fungi growth are determined. Then, relating to the matrix accelerated carbonation and leaching operation are performed to reduce matrix pH. Different ways of incubation are also studied.

2. Materials and methods

2.1. Matrix preparation

We focus on a model system: a pure cement paste. This study was conducted with white Portland cement CEM I 52.5 R. The water/cement mass ratio is 0.55. Hardened cement paste samples are parallelepipedic shaped, $1 \times 2.5 \times 8.5$ cm. Samples were demolded 24 h after elaboration and stored 28 days at 100% relative humidity, at room temperature. Samples are sterilized by γ -radiation (30kGy) after 28 days conservation cure or after carbonation.

2.2. Accelerated carbonation operation

Carbonation operation is carried out in conditioning chamber under pure CO₂ flow. Relative humidity is maintained at $65 \pm 5\%$ with salt solution of K₂CO₃ (RP Normapur). In order to monitor the depth of carbonation, freshly broken samples of hydrated cement paste were sprayed with phenolphthalein indicator solution, which changes from colourless below a pH value of approximately 9.2 to purple above this pH value (RILEM 1989). Some samples are dried 24 h at 40°C before carbonation.

2.4. Leaching operation

Leaching operation is performed in 10 litres containers. Reactor solution is continuously replaced with deionized water. Flow rate is 7 ml/min. 3 carbonated specimens are dived into the reactor. Samplings of reactor solution are performed in order to measure calcium and carbonates concentration by ionic chromatography. pH is continuously measured.

2.5. Fungi

2.5.1. Culture media

Alternaria alternata Fries von keissler is obtained from DSMZ collection. Cultures are done on V8 juice agar medium (CaCO₃ 3g/L, Agar 20g/l, V8 juice 200ml/l), and Czapek-Dox modified medium (cellulose powder 30g/l, KNO₃ 3 g:L, MgSO₄·7 H₂O 500 mg/l, KCl 500 mg/l, FeSO₄·7 H₂O 10 mg/l, K₂HPO₄ 1 g/l, agar 20 g/l), to promote sporulation.

Aspergillus niger strain is DSM N°823, obtained from DSMZ collection. Culture are performed on Potatoes Dextrose Agar medium (potatoes dextrose agar broth at 39 g/l). pH is adjusted at 7.2 with NaOH or HCl 0.1M solutions. Media are autoclaved 20 min at 121°C.

2.5.2. Culture condition

Alternaria alternata is cultured at $24 \pm 1^\circ\text{C}$, and $90 \pm 5\%$ relative humidity. Some cultures are exposed to the light of horticultural neon tubes 10h/day in order to increase sporulation. *Aspergillus niger* is cultured at $24 \pm 1^\circ\text{C}$, and $75 \pm 5\%$ relative humidity.

2.5.3. Spore suspension

Spore suspension is performed with spores harvested 21 days for *Alternaria alternata* and 10 days for *Aspergillus niger*. Spores were harvested by flooding the plates with distilled saline solution (9g/l) and lightly scraping the surface. The resulting spore suspension was filtered through cheesecloth.

2.6. Incubation

Two ways of incubation are tested. Specimens are disposed in sterilized boxes (9.5 x 9.5 x 9.5 cm) as shown in figure 1. In the first way, specimens are covered by a thin agar-agar film whereas, in the second one, specimens are soaked with liquid nutritive medium. Then specimens are inoculated with spore suspension. Non carbonated, carbonated and carbonated leached specimens were inoculated.

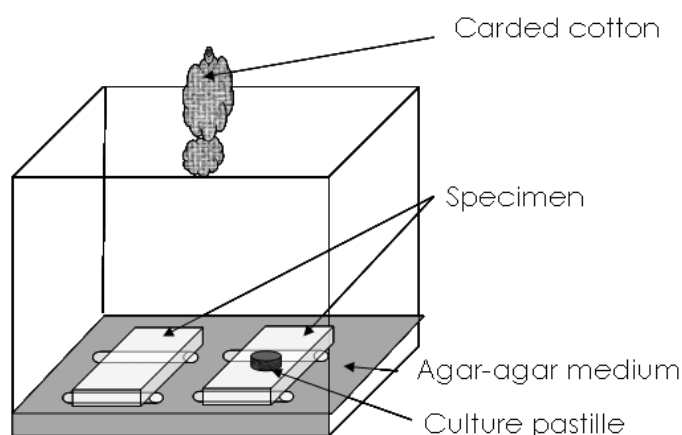


Figure 1. Inoculation

Boxes are kept in culture chamber at $24 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ or $90 \pm 5\%$ HR, respectively for *Aspergillus niger* and *Alternaria alternata*.

3. Results and discussion

3.1. Accelerated carbonation operation

Carbonation rate is maximum for relative humidity comprised between 50 and 65% (Chaussadent 1997). So, to optimize carbonation, experiment is performed with K_2CO_3 solution. Relative humidity was maintained at $65 \pm 5\%$. K_2CO_3 appears to be a suitable salt to control relative humidity during carbonation.

After 48 hours of carbonation, phenolphthalein test revealed a carbonated zone of less than 1 mm for specimens not dried. Specimens dried have been carbonated on 2-3 mm thick (figure 2). However, it must be noticed that carbonation zone is not homogeneous : carbonation is most important on laitance face. Our aim is to reduce pH surface only, so carbonation depth obtained after 48 hours is appropriated.

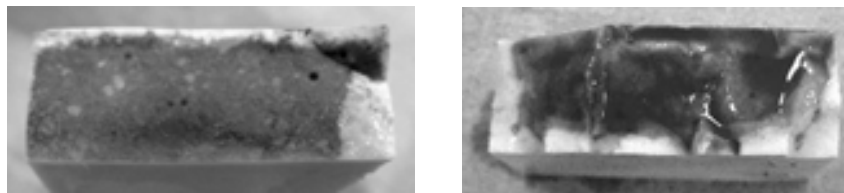


Figure 2. Revelation of carbonated zone (in white) by phenolphthalein test. (a) Specimen not dried. (b) Specimen dried.

3.2. Leaching operation

We can decompose the pH curve in 3 parts :

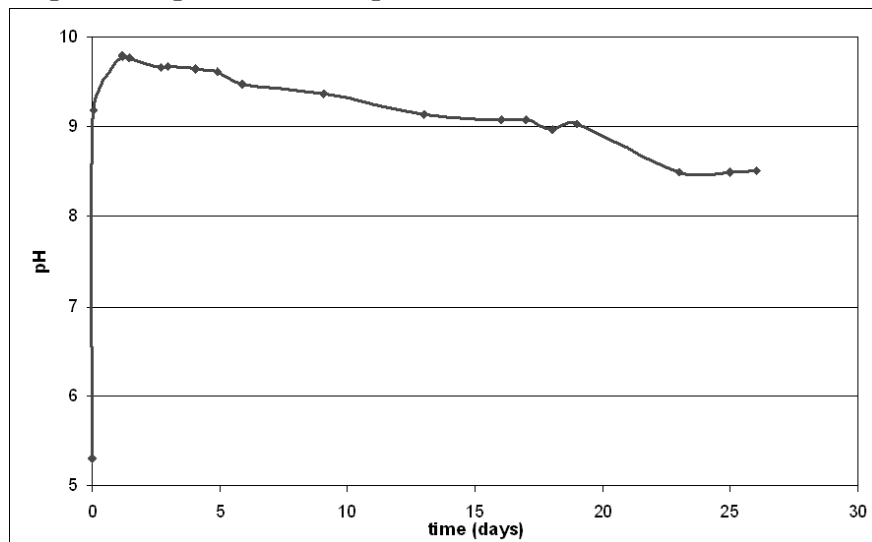


Figure 3. pH evolution of leaching solution

(i) From 0 to 1.5 days : we can note an important increase of pH from 5.3 to 9.8. When specimens are dived, compounds, such as ettringite and portlandite, present on specimen surface are leached. It results in release of alkaline species and an increase of pH.

(ii) 1.5 days to 23 days : we observe a slow and constant decrease in pH, from 9.8 to 8.5. We can assume that once soluble surface compounds are leached, another mechanism takes

place : alkaline species released stem from pore solution. This is due to a concentration gradient between pore solution and leaching solution.

(iii) From 23 days : pH seems to reach a plateau around 8.5. Carbonates present in the leaching solution with calcium released involve calcite precipitation in pore matrix. This phenomenon acts as barrier which limits exchange between pore solution and leaching solution. Equilibrium of dissolution/precipitation of calcite is established, so that we can't obtain a lower pH.

3.3. Incubation

3.3.1. Incubation test for *Alternaria alternata*

First we notice that *Alternaria* growth on agar-agar medium is slow and less dense than in plate culture. Then we don't observe any development of the strain all over the test time for incubation with soaked specimens. Regarding to specimens covered with agar-agar film, no development is observed on non-carbonated and carbonated specimens. On the other hand, 10 days after inoculation, we notice a development on carbonated leached specimens, triggered by the presence of agar-agar medium (figure 4.). After 3 months of incubation, no more development than after 10 days is observed. So those short time tests of incubation stress 3 important points : (i) firstly, we can see that development is possible only on leached specimens, that is to say pH surface is a key parameter to develop our accelerated biodeterioration test. (ii) Then, way of incubation (soaked specimens or with thin agar-agar film) is fundamental to trigger strain growth. (iii) finally, *Alternaria alternata* is a strain very sensitive to experimental parameters including pH, so it isn't the more effective strain to set up rapidly our laboratory test.

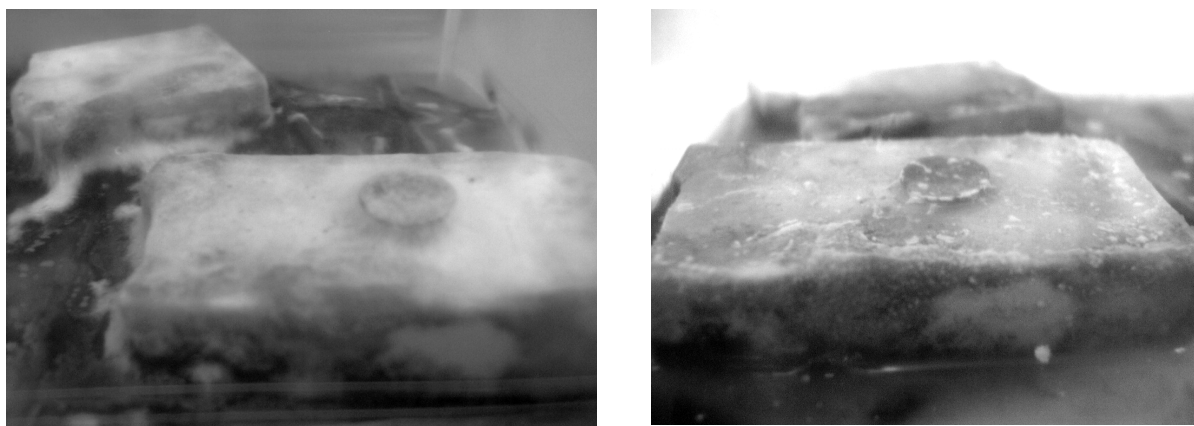


Figure 4. Observations of incubation test with agar-agar film for *Alternaria alternata* at (a) 10 days and (b) 3 months of incubation on leached specimen

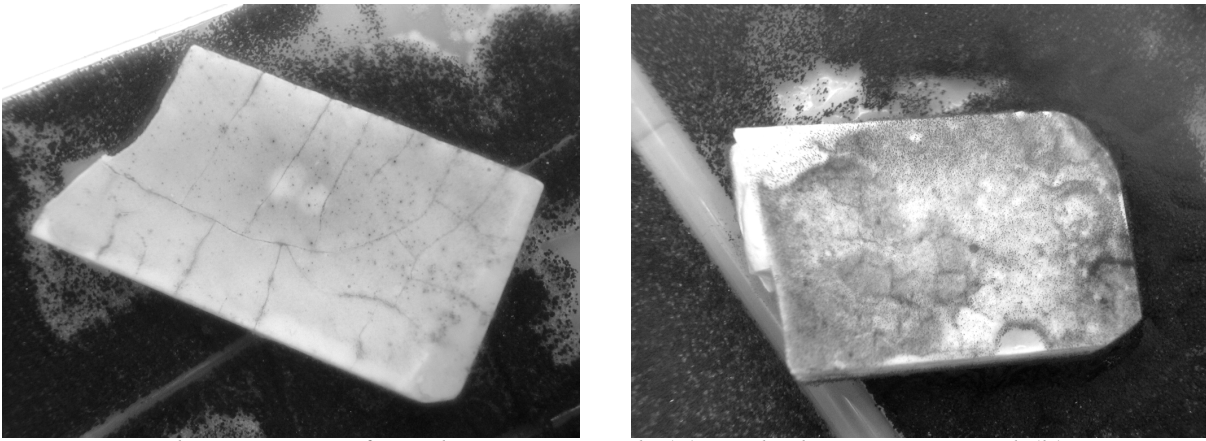


Figure 5. Observations of incubation test with (a) soaked specimens and (b) agar-agar film for *Aspergillus niger* at 10 days of incubation on leached specimens

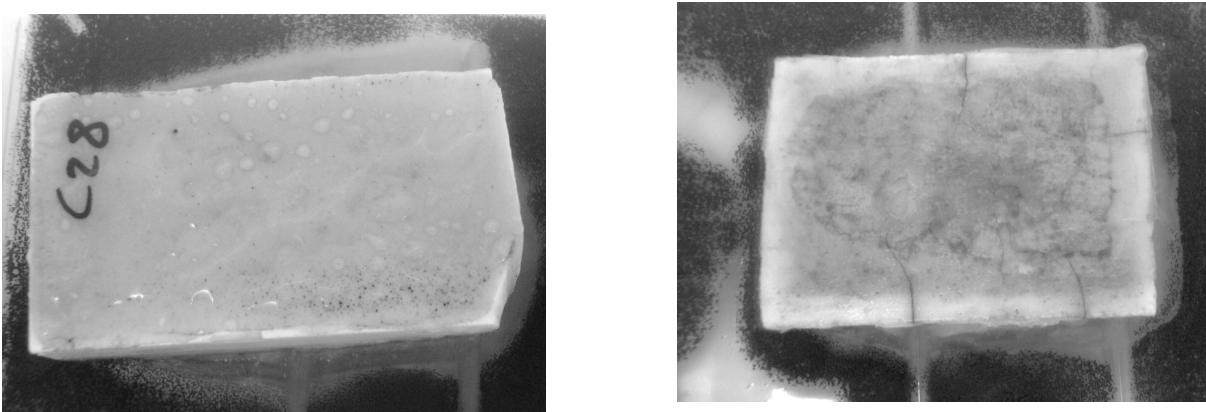


Figure 6. Observations of test with (a) soaked specimens and (b) agar-agar film for *Aspergillus niger* at 10 days of incubation on non carbonated specimens

3.3.2. Incubation test for *Aspergillus niger*

Contrary to previous strain, *Aspergillus niger* growth on agar-agar medium in the box is very good : rapid and dense. Regarding to non carbonated specimens, once again, no development is observed on specimens, no matter the way of inoculation used (figure 5). Concerning carbonated specimens, no growth on soaked specimen is noticed, but for 2/3 of samples development started after 10 days of incubation on agar-agar film. Most interesting result is obtained for carbonated leached specimen. For 1/3 of soaked specimens strain growth is observed after 10 days of incubation. For all specimens with agar-agar film development is very well triggered after 10 days (figure 6). This strain is less sensitive to pH surface than *Alternaria alternata*, that results in a better development on specimens. Absence of growth on non carbonated specimens confirms that pH surface is the key parameter for test set up. These results must be confirmed at 3 months.

3.4 Discussion

Weathering matrix by carbonation and leaching appears as a key parameter for the setting up. As shown on incubation tests, carbonation isn't sufficient to trigger a good development of strains. XRD analyses show that carbonated specimens contain ettringite and portlandite, which mean that carbonation isn't complete. After leaching operation, we characterize only crystalline phases aragonite and calcite (polymorphs of calcium carbonate) by DRX

analyses. We can assume that probably a longer carbonation may avoid leaching operation which necessitate about one month of experiment.

Incubation tests with two different strains show us the importance of pH matrix for the test. Whereas some strains like *Aspergillus niger* are less sensitive with respect to pH, they still can't growth on basic surface (pH 12-13) such as cement.

Then, we can assume that *Aspergillus niger* biodeterioration will be faster than *Alternaria alternata*'s one. Hence, it grows faster and chemical deterioration by acid production necessitates less time to be noticed.

4. Conclusion

Our aim is to set up a laboratory accelerated test in order to have a better understanding of biodeterioration mechanisms. The more complex stage is to obtain in some months the same effects produced in some years in natural environment, that is to say in term of matrix weathering and fungal development. Incubation tests performed on non weathered and weathered specimens (figures 5-6) well underline the importance of pH surface on fungi development.

Once pH matrix resolved, we focus on incubation conditions. It appears that the best way to trigger fungi development is to cover carbonated and leached specimens (figures 4-5) with agar- agar film. We see that further settings are needed for slow development strain as *Alternaria alternata*.

According to preliminary results we can conclude that pH remains the main parameter to control. Best solution for the moment appears to be carbonation and leaching of specimens for matrix weathering and inoculation with spores suspension of specimen covered by a thin agar-agar film.

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